pH/Temperature-Responsive Polymer Composed of Poly((*N*,*N*-dimethylamino)ethyl methacrylate-*co*-ethylacrylamide)

Soon Hong Yuk,* Sun Hang Cho, and Sang Hoon Lee

Advanced Materials Division, Korea Research Institute of Chemical Technology,
P.O. Box 107, Yusung, Korea 305-600

Received May 27, 1997; Revised Manuscript Received September 4, 1997⊗

ABSTRACT: The pH/temperature-induced phase transition of poly((*N*,*N*-dimethylamino)ethyl methacrylate (DMAEMA)-*co*-ethylacrylamide (EAAm)) was investigated. Although polyDMAEMA and polyEAAm were reported to exhibit a lower critical solution temperature (LCST) at 50 and 80 °C, respectively, a LCST shift from 50 to 4 °C was observed with copolymers of DMAEMA with EAAm, and this behavior was quite different at pH 4.0 and 7.4. This is due to the formation of hydrogen bonding between DMAEMA and EAAm residues with a hydrophobic contribution to the LCST. To apply this polymer system to glucose-controlled insulin release, a molded matrix consisting of poly(DMAEMA-*co*-EAAm), glucose oxidase, and insulin was prepared. This matrix exhibited a rapid change from insolubility to solubility when exposed alternately to solutions with ions and a high glucose concentration, resulting in glucose-controlled insulin release.

Introduction

Much interest has been focused on polymer systems that show a phase transition in response to external stimuli such as temperature,[1,2] pH,[3,4] ionic strength,[5] and electric potential[6] because of their scientific or technological importance. Recently, polymer systems that demonstrate a phase transition in response to more than one variable, in particular temperature and pH,[7−9] have been investigated. Feil et al. reported on the mutual influence of pH and temperature on the swelling of ionizable and thermosensitive hydrogels.[7] Later, Chen and Hoffman studied graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH.[8] For both cases, *N*-isopropylacrylamide was used as the temperature-sensitive component and ionic monomers such as (*N*,*N*-diethylamino)ethyl methacrylate or acrylic acid were used as the pH-sensitive component. In our previous report,[9] a pH/temperature-sensitive polymer system with transitions resulting from polymer−water and polymer−polymer interactions has been demonstrated using poly((*N*,*N*-dimethylamino)-ethyl methacrylate (DMAEMA)-*co*-acrylamide (AAm)).

In this study, we propose a new polymer system, which exhibits more significant pH/temperature responsiveness compared to that of poly(DMAEMA-*co*-AAm), and show that glucose-controlled insulin release can be achieved with this polymer system. For this purpose, copolymers of DMAEMA and ethyl acrylamide (EAAm) were prepared and characterized as a function of the copolymer composition. Insulin release in response to glucose was observed.

Experimental Section

Materials. DMAEMA monomer, ammonium persulfate (APS), and tetramethylethylene diamine (TEMED) were purchased from Aldrich. Bovine insulin, *N*,*N*-azobis(isobutyronitrile) (AIBN), and glucose oxidase (GOD) were purchased from Sigma Chemical Co. DMAEMA monomer was distilled before use. Other reagents were used as received.

Synthesis. EAAm was synthesized in our laboratory as described previously.[10] Poly(DMAEMA-*co*-EAAm) was prepared by free radical polymerization as follows: 7.8 g of distilled monomers (mixture of DMAEMA and EAAm) and 0.02 g of AIBN as an initiator were dissolved in 100 mL of water/ethanol binary solvent (5/5 by volume). The feed compositions for copolymers are shown in Table 1. The ampule containing the solution was sealed by conventional methods and immersed in a water bath held at 75 °C for 15 h. After polymerization, all polymers were dialyzed against distilled-deionized water at 4 °C and freeze-dried.

Table 1. Feed Composition for Copolymers in the Study

	DMAEMA		EAAm		
code	amt, g	concn, mol %	amt, g	concn, mol %	$10^{-4}M_w{}^a$
polyDMAEMA	14.2	100			2.8
copolymer I	11.4	80	1.9	20	1.3
copolymer II	8.5	60	3.9	40	2.4
copolymer III	7.1	50	4.9	50	2.9

[a] Measured by laser scattering.

Transmittance Measurements. The phase transition was traced by monitoring the transmittance of a 500 nm light beam on a Spectronic 20 spectrophotometer (Baush & Lomb). The concentration of the aqueous polymer solution was 5 wt %, and the temperature was raised from 15 to 70 °C in 2-deg increments every 10 min. To observe their pH/temperature dependence, the phase transitions of polymers in citric−phosphate buffer solution versus temperature at two pH values (4.0 and 7.4) were measured.

FT-IR Measurement. For Fourier transform infrared (FT-IR) measurement, thin films of polymers were cast from 0.5 wt % distilled-deionized water onto separate CaF_2 plates at room temperature. Most of the water in the films was removed by evaporation at 50 °C in a vacuum oven for 24 h. FT-IR spectra of the dried polymer were measured on a Magna IR spectrophotometer (Nicolet Inc., Madison, WI) using 64 average scans at a resolution of 4 cm^{-1}.

Preparation of Insulin-Loaded Matrix. Lyophilized copolymer was ground down to colloidal dimensions (<1 μm) using a laboratory planetary mill (Pulverisette, Fritsch GmbH, Germany). A 110 mg sample of copolymer powder, 20 mg of bovine insulin, and 20 mg of GOD were mixed, and the mixture was compressed into a disk-shaped matrix of 5-mm thickness and 15-mm diameter.

Measurement of Weight Loss of Insulin-Loaded Matrix in Response to Glucose. After immersion in phosphate buffer solution (PBS) for a desired time at 37 °C, the insulin-loaded matrix was removed and dried in a vacuum oven at room temperature. The percent of weight of the matrix was determined as a function of time.

---

* To whom correspondence should be addressed.

⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1997.

S0024-9297(97)00725-0 CCC: $14.00

Table 2. Curve-Fitting Results of the N−H Stretching

	non-hydrogen-bonded N−H			hydrogen-bonded N−H			amide B		
code	ν, cm^{-1}	$W_{1/2}$, cm^{-1}	area	ν, cm^{-1}	$W_{1/2}$, cm^{-1}	area	ν, cm^{-1}	$W_{1/2}$, cm^{-1}	area
polyDMAEMA	3408.6	144.8	0.7	3311.2	246.9	2.7	3177.3	218.2	2.5
copolymer I	3390.3	114.5	0.4	3325.4	60.7	0.1	3272.5	135.8	0.9
copolymer II	3393.8	124.4	1.2	3312.1	104.2	1.1	3208.9	60.8	0.2
copolymer III	3393.8	159.0	1.6	3287.9	165.6	4.1	3204.8	65.7	0.1

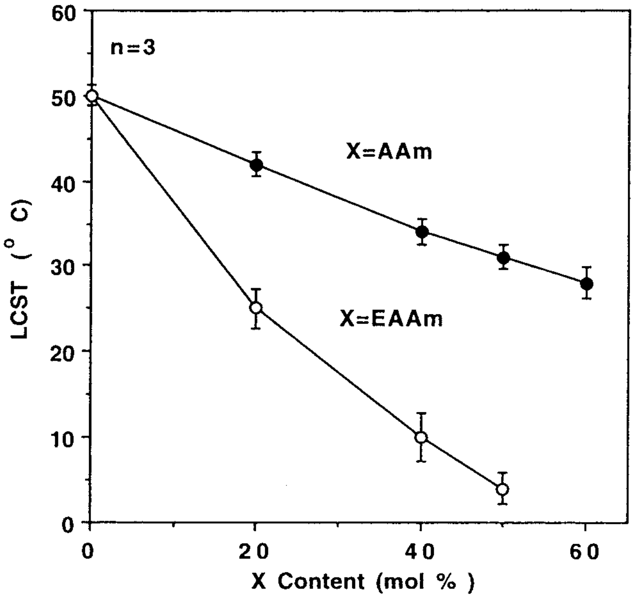

Figure 1. LCST of poly(DMAEMA-*co*-X) in water as a function of X content. (X = AAm, reported value from ref 9.)

Release Experiment. Insulin release from the insulin-loaded matrix was measured in response to alternating changes of glucose concentration when 150 mg of an insulin-loaded matrix was introduced to 100 mL of PBS at 37 °C. The amount of released insulin was measured by taking 1 mL of the release medium at a specific time and immersing the matrix in a fresh release medium. Insulin was determined by reversed-phase HPLC, using a Resolvex C_{18} (Fisher Scientific) and 0.01 N HCl/acetonitrile (80/20−50/50, v/v %) mobile phase over 30 min at a flow rate of 1 mL/min. The eluate was monitored by optical absorption at 210 nm.

Results and Discussion

pH/Temperature-Induced Phase Transition. As reported previously,[9,11] polyDMAEMA and polyEAAm have lower critical solution temperatures (LCST)s at 50 and 80 °C, respectively. Interestingly, poly(DMAEMA-*co*-EAAm) exhibits the LCST between 0 and 50 °C depending on the copolymer composition.

The effect of the EAAm content on the LCST in water is shown in Figure 1. When the temperature of a polyDMAEMA aqueous solution was raised above 50 °C, the polymer precipitated from the solution. This is due to the hydrophobic interaction between (*N*,*N*-dimethylamino)ethyl groups above the LCST. With the incorporation of EAAm in the copolymer, the LCST was shifted to a lower temperature. In general, the LCST should increase with increasing hydrophilicity of the polymer.[12] However, a LCST shift to a lower temperature was observed with the incorporation of the hydrophilic EAAm. In addition, the LCST shift of poly(DMAEMA-*co*-EAAm) is larger than that in the poly(DMAEMA-*co*-AAm).[9]

FT-IR studies were used to characterize the intra/intermolecular interaction in the copolymer. Figure 2 shows characteristic absorption bands of copolymers in the N−H stretching region. In copolymers of EAAm with DMAEMA, a rather broad band centered at approximately 3300 cm^{-1} is observed, which is assigned to ethylamide groups that are hydrogen bonded to DMAEMA. The absorbance at approximately 3300 cm^{-1} increases with the EAAm content in the copolymer. (According to Coleman et al.,[13] the spectrum of ethylurethane is characterized by a rather broad band centered at approximately 3340 cm^{-1}, which is assigned to N−H groups that are hydrogen bonded to C=O groups. The non-hydrogen-bonded N−H stretching is barely detectable as a weak shoulder at about 3440 cm^{-1}.) The disubstitued amide is known to be a powerful hydrogen bond acceptor.[14] As for the unsubstituted amide such as AAm, its role as hydrogen bond donor has been demonstrated.[15] Therefore, ethylamide, a monosubstituted amide, can be a hydrogen bond donor or acceptor depending on the environment. Because the dimethylamino group in DMAEMA is known to be a powerful hydrogen bond acceptor,[16] it is reasonable to suggest efficient hydrogen bondings between ethylamide and *N*,*N*-dimethylamino groups.

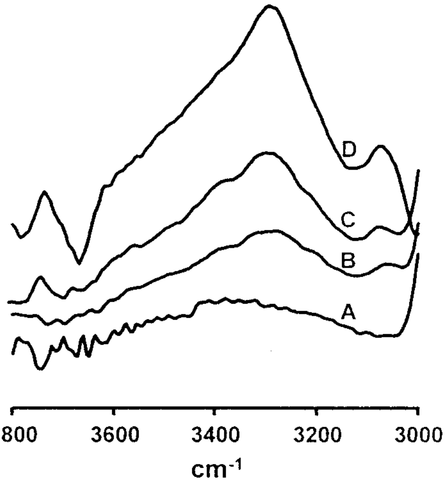

Figure 2. N−H stretching region of the infrared spectrum of (A) polyDMAEMA, (B) copolymer I, (C) copolymer II, and (D) copolymer III.

Using a curve-fitting procedure that has been previously described,[17] we resolved the N−H stretching region into three components. Two of the curves correspond to the non-hydrogen-bonded and hydrogen-bonded N−H stretching modes, and the third is attributed to the amide B mode.[18] Table 2 presents curve-fitting results of the non-hydrogen-bonded and hydrogen-bonded bands. With the increase of the EAAm content in the copolymer, the area ascribed to the hydrogen-bonded N−H stretching band increases significantly. Conversely, the area of the non-hydrogen-bonded N−H stretching band remains relatively constant. These results strongly suggest efficient hydrogen bonding between ethylamide and *N*,*N*-dimethylamino groups.

From these results, it can be concluded that the LCST shift to the lower temperature is due to the formation of hydrogen bonds, which protect (*N*,*N*-dimethylamino)ethyl groups from exposure to water and result in a

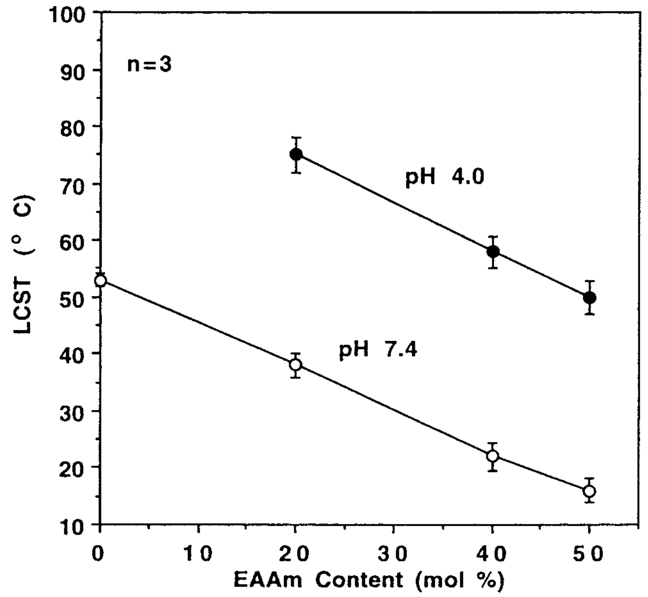

Figure 3. LCST of poly(DMAEMA-*co*-EAAm) in citric–phosphate buffer solution at pH 4.0 and 7.4.

hydrophobic contribution to the LCST. Because of a more hydrophobic nature of ethylamide, poly(DMAEMA-*co*-EAAm) exhibits a more significant temperature responsiveness than poly(DMAEMA-*co*-AAm).

The temperature-induced behavior of copolymers at two pHs (4.0 and 7.4) is observed as shown in Figure 3. At pH 4, no LCST was observed with polyDMAEMA and the LCST of all copolymers was increased compared to that at pH 7.4. At pH 4.0, (*N*,*N*-dimethylamino)ethyl groups of DMAEMA are fully ionized. An increasing electrostatic repulsion between charged sites on DMAEMA disrupts the hydrogen bonds between EAAm and DMAEMA. These interfere with the hydrophobic interactions between (*N*,*N*-dimethylamino)ethyl groups above the LCST and the hydrophobic contribution to the LCST due to hydrogen bonding.

Glucose-Controlled Insulin Release. Figure 4 is a schematic description of the glucose-controlled insulin release using poly(DMAEMA-*co*-EAAm). In the presence of glucose, gluconic acid generated by the glucose–GOD reaction protonates dimethylamino groups of poly(DMAEMA-*co*-EAAm), inducing the LCST shift to higher temperature from the surface of the insulin-loaded matrix. This leads to the disintegration of the matrix with polymer dissociation from the surface with the release of insulin. Based on this concept, an insulin-loaded matrix was designed and characterized. Copolymer III was selected as a model polymer for the preparation of an insulin-loaded matrix cosidering its pH/temperature responsiveness. Figure 5 shows the weight loss of the matrix in PBS at two different glucose concentrations. We found that 100% of the initial weight had been lost at 5 g/L of glucose concentration during 24 h, whereas 10% of the weight had been lost at 0.5 g/L of glucose concentration. These results indicate that this polymer system responds to the change of glucose concentration in the presence of GOD.

Figure 6 shows the alternating insulin release rate in response to an alternating exposure of the insulin-loaded matrix to high and low aqueous glucose solutions. Minimal release was observed at the lower concentration of glucose. The large deviation in the release rate was attributed to inhomogeneous mixing of the components in the insulin-loaded matrix causing its irregular dissolution. The release rate was not maintained constantly from cycle to cycle. As the release experiment was continued, the matrix swelled from the surface and this led to the rapid disintegration of matrix, resulting in the increase of release rate. At the later stage of the release experiment, the decreased amount of insulin in the matrix after cycles affected the decreasing release rate.

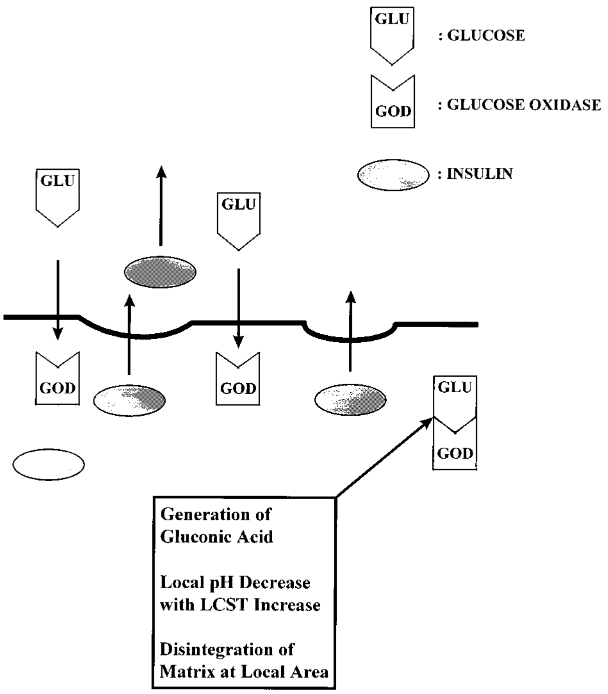

Figure 4. Schematic representation of the glucose-controlled insulin release using poly(DMAEMA-*co*-EAAm).

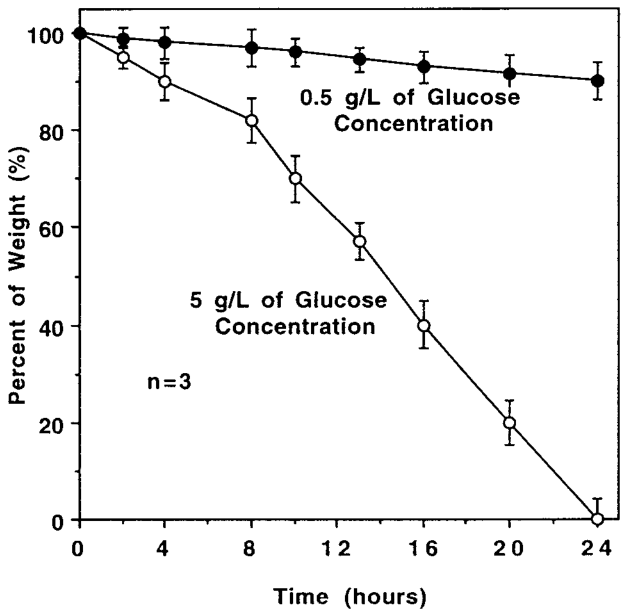

Figure 5. Disintegration of the polymer matrix in phosphate buffer solution at two glucose concentrations.

Conclusions

pH/temperature-responsive polymers with functions resulting in polymer–water and polymer–polymer interactions were prepared and a transition mechanism was proposed. A hydrophobic contribution to the LCST caused by the hydrogen bond is a major factor in controlling the phase transition. A glucose-controlled

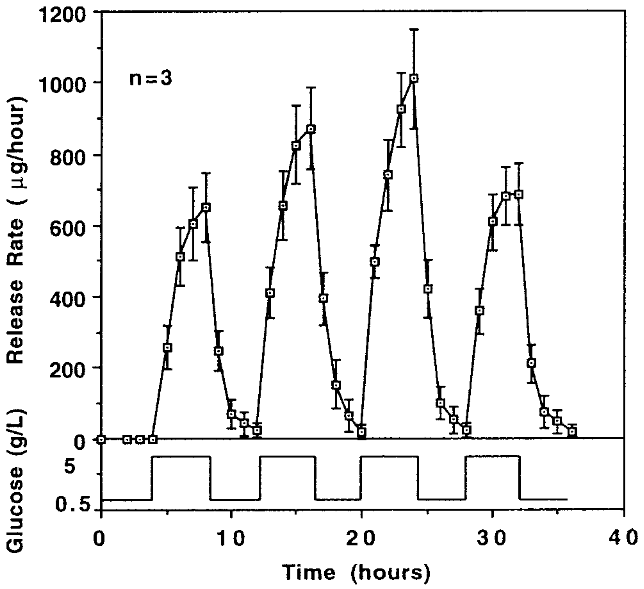

Figure 6. Insulin release from the insulin-loaded matrix in response to alternating change of glucose concentration.

insulin release system was demonstrated. Although this work is still far from a practical application, the concepts used may lead to the design of a self-regulated insulin delivery system implanted into the peritoneal cavity by considering polymer toxicity and formulating a material with desirable properties.

References and Notes

(1) Bae, Y. H.; Okano, T.; Kim, S. W. *J. Polym. Sci., Polym. Phys.* **1990**, *28*, 923.
(2) Hoffman, A. S. *J. Controlled Release* **1986**, *4*, 213.
(3) Kopecek, J.; Vacik, J.; Lim, D. *J. Polym. Sci., Polym. Chem. Ed.* **1971**, *9*, 2801.
(4) Siegel, R. A.; Firestone, B. A. *Macromolecules* **1988**, *21*, 3254.
(5) Ricka, J.; Tanaka, T. *Macromolecules* **1984**, *17*, 2916.
(6) Kwon, I. C.; Bae, Y. H.; Kim, S. W. *Nature* **1991**, *354*, 291.
(7) Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. *Macromolecules* **1992**, *25*, 5528.
(8) Chen, G.; Hoffman, A. S. *Nature* **1995**, *373*, 49.
(9) Cho, S. H.; Jhon, M. S.; Yuk, S. H.; Lee, H. B. *J. Polym. Sci., Part B: Polym. Phys.* **1997**, *35*, 595.
(10) McCormick, C. L.; Nonaka, T.; Johnson, C. B. *Polymer* **1988**, *29*, 731.
(11) Mueller, K. F. *Polymer* **1992**, *34*, 3470.
(12) Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. *Macromolecules* **1993**, *26*, 2496.
(13) Coleman, M. M.; Shrovanek, D. J.; Hu, J.; Painter, P. C. *Macromolecules* **1988**, *21*, 59.
(14) Su, C. W.; Watson, J. W. *J. Am. Chem. Soc.* **1974**, *96*, 185.
(15) Ilman, F.; Tanaka, T.; Kokufuta, E. *Nature* **1991**, *349*, 400.
(16) McCormick, C. L.; Blackmon, K. P.; Elliott, D. L. *Polymer* **1986**, *27*, 1976.
(17) Coleman, M. M.; Lee, K. H.; Shrovanek, D. J.; Painter P. C. *Macromolecules* **1986**, *19*, 2149.
(18) Moore, W. H.; Krimm, S. *Biopolymers* **1976**, *15*, 2439.

MA970725W